EXHIBIT 2

Docket No.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	The University of Warwick
Serial No.	10/535,433
Filed	02/02/2006
Title	Antibody Secretion
Art Unit	643
Examiner	Bristol, Lynne Anne

DECLARATION OF

UNDER 37 CFR § 1.132

Honorable Commission of Patents

And Trademarks

Washington, DC 20231

Sir:

I, Lorenzo Frigerio, hereby declare and state as follows:

1. I received a Masters of Science degree (Italian Laurea) in 1991 from The Universita' Cattolica, Piacenza, Italy
2. In 1995, I received a degree of Doctor of Philosophy in Plant Genetics from The Universita' Cattolica, Piacenza, Italy
3. I am currently employed at the University of Warwick
   My Curriculum Vitae is attached (annex 1).
4. I have been provided with a copy of the above United States Patent Application, as well as the Office Actions issued by the USPTO and prior art cited by the Examiner. I have carefully reviewed the application and the related prosecution documents, including the Response and revised claims submitted on December 23, 2008. The claimed invention relates to a method of making an antibody molecule, the antibody molecule containing an immunoglobulin heavy chain comprising a $\alpha3$ domain or a mu domain, the method comprising providing a nucleotide sequence encoding an

immunoglobulin heavy chain molecule, modifying the region encoding C-terminal 18 amino acids to remove or reduce the effectiveness of one or more vacuolar targeting signals of the encoded immunoglobulin, inserting the modified nucleotide sequence into a plant host cell; and causing the host cell to express the modified nucleotide sequence with co-expression of antibody light chains to form a modified immunoglobulin heavy chain from the host cell.

5. I understand that the US Patent Office has asserted that the description outlined in the specification is insufficient to enable one skilled in the art as of the filing of this application to make and use the method claimed.

6. In my opinion, the method of the invention can be practiced in its full scope using the teachings in the application together with techniques that were already used in the art to produce antibodies in plants. Methods of producing antibodies are generally known in the art and indeed are exemplified in US 6,417,429 and WO 97/42313 which are specifically cited in the application as filed. This is further evidenced by the attached paper by Hiatt et al Nature (1989) vol 342 76-78. The method of the invention modifies a small, defined region of the heavy chain of an antibody that is the last 18 C-terminal amino acids of the heavy chain.

7. I believe that at the filing date of the application recombinant antibody technology was well characterised and given the general knowledge of antibody structure and function, once the region to be modified had been identified by the present invention, practicing the full scope of the invention could be predicted from the teaching of the application with reasonable certainty, and without any undue experimentation.

8. The claims are limited to methods using antibodies having a $\alpha3$ domain or a mu domain that is comprising a $\alpha3$ domain of IgA or mu domain of IgM. In my opinion one skilled in the art would understand what these domains are and where they are found in IgA and IgM antibodies. Indeed I note that the application as filed gives the C-terminal sequences of a large number of publicly available sequences of IgM and IgA heavy chains showing the conserved nature of many of these chains (see Figure 8). Such IgA and IgM antibody sequences are generally well known in the art.

9. I note that the pending claims relate to a method of making an antibody containing such antibodies. The Examiner indicates that the structural relationship of the antibodies is not in some way defined. However, I believe that the method clearly defines the starting material (the unmodified antibody) and clearly indicates the way that the antibody is modified by specifically defining the region to be modified, that is

the last 18 amino acids of the C-terminal chain. Hence the structural feature needed to be modified is explicitly defined in the claim. Moreover, the application as filed provides further structural information about the nature of the vacuolar targeting signal, that is, for example, the consensus sequence shown in claim 36. I therefore believe that the structure modified is fully defined both by position and structure and would be understood by one skilled in the art.

10. I believe that such a person of ordinary skill in the art would be of relatively high skill, such as a master's degree in biology.

11. I believe that the application as filed gives details of how to determine, without undue experimentation, how to identify whether the heavy chain is being degraded in vacuoles and whether it is secreted. I understand that the enclosed flow diagram (Exhibit A) showing the steps taken to produce and assay for the claimed method has been presented to the Examiner. Such methods are routine and do not involve undue experimentation. Moreover, a comparison between unmodified and modified heavy chain is readily carried out and indeed is exemplified in the application as filed (see for example Figures 5, 7, 9 and 10 of the application as filed). I believe that this is fully reproducible by the skilled person and would not be unreasonable.

12. I believe that methods used to modify a limited, defined region of the nucleotide sequence, such as a region encoding the C-terminal 18 amino acids of a protein, are per se well known in the art. The application explicitly gives examples of modifying the region; (i) deletion to produce the construct ΔC18 and (ii) replacing the deleted region with a modified sequence (for example – PAAAAACY), the results of which are shown in Figures 7, 9 and 10.

13. Page 5, line 13 to page 6 line 2 of the PCT application as filed gives a number of alternative methods of removing or reducing the vacuolar targeting signal. I believe that modifying such a limited region of a protein was generally well known in the art and each of the alternatives were generally well known in the art. The article by Matsuoka and Neuhaus, referred to in that section gives further details of how to modify vacuolar starting signals. Systems for mutagenising and introducing mutations have been available for many years. Indeed, one of the standard techniques in the field utilises the "Quick Change" system for Stratagene. This has been commercially available since at least 2001, as evidenced from the penultimate paragraph on page 173 of my enclosed paper (Frigerio et al), (2001) Plant Physiol,

126 167-175. Hence, such methods would be readily understood by the skilled person.

The antibody construct used as the unmodified antibody is an IgG/A hybrid which was known in the art at the time of filing to bind Streptococcal SAI/II antigen.

14. I understand that the Examiner queries whether the application demonstrates the ability to bind target antigen. I note that this is not a requisite of the current claims. However, I believe that assays for antigen binding were generally known in the art at the time the application was filed. The modifications to the C-terminus of the antibody heavy chain do not affect antigen binding at the N-terminus end. For example, the Examiner will recall the Fab fragments of antibodies, generated by papain, still have antigen binding, without the C-terminus end of the antibody.

15. The application as filed and prior art acknowledged in the application, for example WO 2004/046190, shows how to produce a functionally active antibody. The application clearly states that ΔC18, lacking the vacuolar targeting signal, was co-expressed with κ light chains and produced a correctly assembled antibody (see page 22 lines 16-19 of the PCT application). Assays for antibody antigen binding are generally well known and indeed were the subject of many undergraduate courses at that time to my personal knowledge. Hence the skilled person would understand how to assay for antigen binding.

16. The Examiner believes that the application does not disclose antibodies having a utility. However, I believe that, for example, ΔC18 antibodies clearly have that utility. The application explicitly states that functional antibodies were produced. ΔC18 is based on IgA/G which was known to have antigen binding activity. There is no reason to believe that simply deleting the C-terminal 18 amino acids would have any effect on the antigen binding activity of the antibody.

17. I enclose as Annex 2, extra data showing that the ΔC18 deletion and optionally PAAAAACY(PA5CY) addition to that construct produce increased amounts of functional antibody when co-expressed with light chain. This was compared to unmodified IgA/G construct ("G2A"). This is shown in Figure 1. Both of these constructs are expressly exemplified in the current patent application. This is compared to two other constructs expressed in plants with C-terminal sequences (GGGGGCG – "G5CG" and QQQQQCQ – "Q5CQ"), which, whilst in these initial

tests are less successful, they still produce active antigen binding antibodies. The data for these latter two antibodies is still being investigated, but it is believed that the DNA encoding those constructs may not have integrated optimally into the genome of the host plants resulting in decreased levels of expression.

The data obtained in plants was the culmination of a screening programme which originally started with 9 constructs, in addition to the ΔC18 deletion (see Table 1). The data was obtained using the experimental procedures of the instant patent application

Constructs were expressed in a transient expression assay for vacuolar degradation (using the methods described in the patent application). In this assay G2A is indicated as having high levels of degradation (+++). 9 of the 10 constructs showed better levels of degradation (++, + or -). The best constructs marked + or – (ΔC18, PA_5CY, PA_5CG, G_8CY, G8CG, G_5CG and Q_5CG), were used in a transient expression assay. Please note "-" indicates poor expression, +, ++ and +++ increasingly better levels. The best levels of heavy chain expression (ΔC18, PA_5CY, PA_5CG, G_5CG and Q_5CG), were expression in stable transgenic heavy chain plants. One of these, PA_5CG did not produce high levels of heavy chain expression. The reason for this has not yet been identified, but it may be due to the position of the construct encoding the heavy chain not being optimal in the plant. The remaining constructs were crossed with the plants containing constructs encoding light chain and tetrameric antibody was expressed and antigen binding determined as shown in Figure 1.

This confirms that the methods claimed produce antibodies that bind and have increased levels of expression compared to native antibodies.

18. I have read the objections raised by the Examiner indicating that the invention is obvious in view of the prior art cited by the Examiner. The invention is inventive, because it identifies for the first time a negative correlation between vacuolar sorting and the overall yield of antibody (the crucial information being the secretion of the ΔC18 antibody lacking the vacuolar sorting signal), and it indicates a novel strategy to suppress vacuolar sorting.

19. I believe that a skilled artisan would not look for vacuolar sorting signals in antibodies. Antibodies are not native plant proteins. In their native state they are produced in cells that do not have vacuoles. The assumption in this field, before publication of this work, was that non-plant sequences would have been devoid of the sequences. In native systems there is no need for such sequences. Had it been obvious I would have looked for such signals as soon as I found there was a problem producing the antibodies. However, it is only after a systematic analysis of IgA/G degradation in plants, with several other potential reasons for the problem investigated and rejected, that I identified the vacuolar sorting signal. In my opinion the identification of the vacuolar targeting signal cannot be considered to have been be obvious without the benefit of hindsight. It was not predicable from the prior art.

20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Lorenzo Frigerio

Dated 9th September 2009

Annex 1

Curriculum vitae - Lorenzo Frigerio

DOB: 9th November 1967

EDUCATION

1995- Ph.D., Plant Genetics, Universita' Cattolica S.Cuore, Piacenza, Italy

1991 - Laurea (Degree), Agricultural Sciences (grade: 110/110 *cum laude*), Universita' Cattolica S. Cuore, Piacenza, Italy.

ACADEMIC EMPLOYMENT

1999-present – Lecturer, Senior Lecturer, Reader (Associate Professor) in Plant Sciences, Department of Biological Sciences, University of Warwick, UK

1998 - 1999 - Postdoctoral Research Fellow, Department of Biological Sciences, University of Warwick, UK

1997- 1998 - Postdoctoral Research Fellow, Italian National Research Council (CNR), Istituto Biosintesi Vegetali CNR, Milano, Italy.

1995 - 1997 - Postdoctoral Research Fellow (funded by EU Framework IV Human Capital & Mobility), Department of Biological Sciences, University of Warwick, UK

RESEARCH INTERESTS

I have worked on the mechanisms of protein sorting in the plant secretory pathway for 10 years. As a postdoctoral researcher, my work led to the discovery of vacuolar sorting signals for different seed storage proteins. Since establishing my own group at Warwick in 1999, my research has focussed on:
(i) The transport and processing of storage proteins and toxins to plant seed storage vacuoles.
(ii) The elucidation of the quality control mechanisms operating in the plant ER on newly-synthesised proteins.
(iii) The detailed analysis of the intracellular targeting of recombinant monoclonal antibodies in the plant secretory pathway, with the aim to maximise their production.
(iv) The function of plant reticulons.

RECENT GRANTS (as PI)

Source of Funds	Title of Project	Duration (Months)	Starting Date	Total Value
Leverhulme Trust	How are proteins sorted to storage vacuoles in seeds?	36	January 2008	£139,469
Wellcome Trust	Maximising the yield of plant-made immunoglobulin	24	April 2007	£110,485
EU	Pharma-Planta	60	June 2004	€ 350,000

SELECTED PUBLICATIONS (LAST 5 YEARS)

1. Bouchekhima A, Frigerio L, Kirkilionis M (2009). Geometric quantification of the plant endoplasmic reticulum. Journal of Microscopy 234:158-172.

2. Frigerio L , Hinz G, Robinson DG (2008). Multiple vacuoles in plant cells: rule or exception? Traffic 9:1564 – 1570

3. Craddock C, Hunter P, Szakacs E, Hinz G, Robinson D, Frigerio L (2008) Lack of a vacuolar sorting receptor leads to non-specific missorting of soluble vacuolar proteins in Arabidopsis seeds. Traffic 9:408-416

4. Tolley N, Sparkes IA, Hunter PR, Craddock CP, Nuttall J, Roberts LM, Hawes C, Pedrazzini E, Frigerio L (2008) Overexpression of a plant reticulon remodels the lumen of the cortical endoplasmic reticulum but does not perturb protein transport. Traffic 9: 94-102

5. Marshall RS, Jolliffe NA, Ceriotti A, Snowden CJ, Lord JM, Frigerio L, Roberts LM (2008). The role of CDC48 in the retro-translocation of non ubiquitinated toxin substrates in plant cells. J. Biol. Chem. 283:15869-15877

6. Irons SL, Nuttall J, Floss DM, Frigerio L, Kotzer AM, Hawes C (2008). Fluorescent protein fusions to a HIV monoclonal antibody reveal its intracellular transport through the plant endomembrane system. Plant Biotech J 6: 649 – 662

7. Barbante A, Irons S, Hawes C, Frigerio L, Vitale A, Pedrazzini E (2008). Anchoring to the cytosolic face of the ER membrane: a new strategy to stabilize a cytosolic recombinant antigen in plants. Plant Biotech J 9:560-575

8. Hunter PR, Craddock CP, Di Benedetto S, Roberts LM, Frigerio L (2007) Fluorescent Reporter Proteins for the Tonoplast and the Vacuolar Lumen Identify a Single Vacuolar Compartment in Arabidopsis Cells. Plant Physiol 145: 1371-1382

9. Marusic C, Nuttall J, Buriani G, Lico C, Baschieri S, Ma J-KC, Benvenuto E, Frigerio L (2007). Expression and intracellular targeting of HIV nef variants in transiently transformed and transgenic tobacco cells. BMC Biotechnol. 7:12

10. Maggio C, Barbante A, Ferro F, Frigerio L, Pedrazzini E (2007) Intracellular sorting of the tail-anchored protein cytochrome b5 in plants: a comparative study using different isoforms from rabbit and Arabidopsis. J. Exp. Bot. 58:1365-1379

11. Obregon P, Chargelegue D, Drake PMW, Prada A, Nuttall J, Frigerio L, Ma J-KC (2006). HIV-1 p24-Immunoglobulin fusion molecule: A new strategy for plant-based protein production. Plant Biotechnol. J. 4:195-207

12. Nuttall, J, Ma, JK-C, Frigerio, L (2005). A functional antibody lacking N-linked glycans is efficiently folded, assembled and secreted by tobacco mesophyll protoplasts. Plant Biotechnol. J. 3:497-504

13. Jolliffe NA, Brown JC, Neumann U, Vicre' M, Bachi A, Hawes C, Ceriotti A, Roberts LM, Frigerio L (2004). Transport of ricin and 2S albumin precursors to the storage vacuoles of Ricinus communis endosperm involves the Golgi and VSR-like receptors. Plant J. 39:821-833

Annex 2

<u>Comparative data comparing antibodies altered by deleting the last 18 amino acids of the C-terminal end of the heavy chain, and optionally replacing the deleted amino acids with replacement amino acid sequences</u>

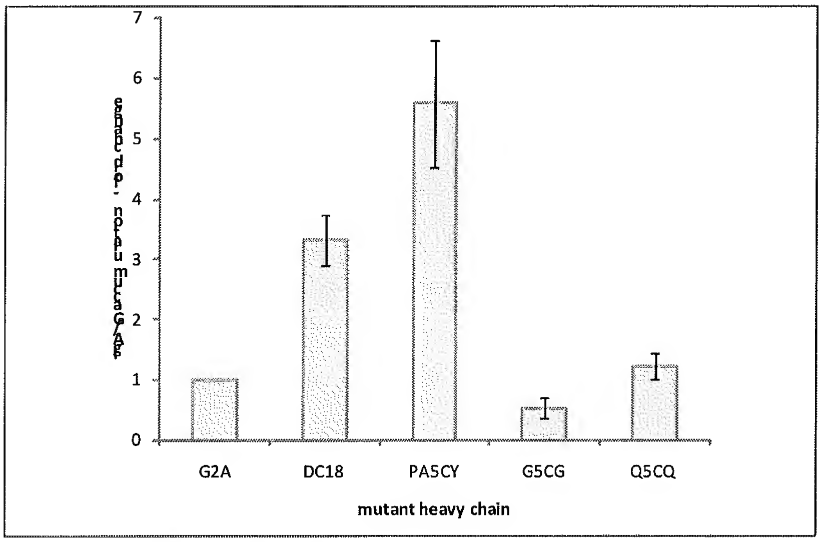

Figure 1. Comparative yield of functional IgA/G in seedlings of transgenic lines carrying mutant heavy chains. The fourth leaves from 20 axenically grown seedlings from each of the indicated heavy x light chain transgenic lines were homogenised and equal amounts of protein loaded onto capture ELISA plates containing immobilised streptococcal SAI/II antigen. The average ELISA results are expressed as fold-change compared to the reference construct G2A. Bars indicate standard deviation.

Heavy chain mutant	Transient exp. assay: vacuolar degradation	Transient exp. assay: secretion	Stable heavy chain plants	Crossed with light chain plants	Proceed to validation	comments
Benchmark constructs						
G2A	+++	-	√	√	yes	Original heavy chain
ΔC18	-	+++	√	√	yes	Complete tailpiece deletion
Mutant constructs						
PA_5CY	-	++	√	√	yes	
PA_5CG	-	++	√	-	no	Very low expression

						in stable lines
PACY	++	ND			no	
G_8CY	+	+			no	
G_8CG	+	+			no	
G_5CG	-	++	√	√	yes	
Q_5CQ	-	++	√	√	yes	
HASTPEPDPVACY	+++	ND			no	IgG hinge region
SLLGRPVVPNFNADVCM	++	ND			no	mutated proricin linker

Table 1. Summary of transient expression experiments on mutant heavy chain constructs. The indicated constructs were co-infiltrated into tobacco leaf cells with light chain construct. Vacuolar degradation was assessed by immunoblot and secretion by pulse-chase analysis on protoplasts from infiltrated sectors. Vacuolar degradation and secretion of the mutant heavy chain were scored against the reference constructs G2A and ΔC18, respectively.